Journal of Integrative Agriculture 2017, 16(5): 1112–1119

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Establishment of a tetracycline-off and heat shock-on gene expression system in tobacco

ZHOU You, LI Jin-hua, PAN Yu, ZHENG Yu, PAN Yang-lu, DING Yu-mei, SU Cheng-gang, ZHANG Xing-guo

Key Laboratory of Horticulture Science for Southern Mountainous Regions, Ministry of Education/College of Horticulture and Landscape Architecture, Southwest University, Chongqing 400715, P.R.China

Abstract

The tetracycline (Tet)-off gene expression regulation system based on the TetR-VP16/Top10 construct has not been widely utilized in plants, for its highly expressed TetR-VP16 activator is toxic to some plants and repeatedly replenishing tetracycline to turn off the constitutively active system is a tedious process. To solve these problems, a Tet-off and heat shock (HS)-on gene expression regulation system was constructed in this study. This system is composed of a chimeric transactivator gene *TetR-HSF* that is derived from a Tet repressor (TetR) and a HS transcription factor (HSF) controlled by a HS promoter HSP70m, and a Tet operator containing hybrid promoter, Om35S, that drives expression of the β-glucuronidase (*GUS*) gene. The resultant system yields a GUS expression pattern similar to that of the HSP70m promoter under inducing temperatures and at 35 and 40°C drives GUS expression to a similar level as the *Cauliflower mosaic virus* (CaMV) 35S promoter. Further examination revealed that the *TetR-HSF* and *GUS* genes were induced by HS, reaching peak expression after 1 and 6 h treatment, respectively, and the HS induction of the expression system could be inhibited by Tet. This system will provide a useful tool for transgenic studies of plants in the laboratory and in the field, including transgene function analysis, agronomic trait improvement, biopharmaceutical protein production and others.

Keywords: gene expression system, heat shock-on, tetracycline-off, TetR-HSF transactivator, tobacco

1. Introduction

Tetracycline (Tet)-resistant bacteria possess a gene encoding a transporter protein that shuttles Tet out of the cell. In the absence of Tet, this gene is repressed by Tet repressors (TetRs) that bind to Tet operator (TetO) sequences in the promoter. When Tet is present, it binds with TetR, inducing a conformational change that results in the release of TetR from the promoter and subsequent expression of the transporter gene (Bertrand *et al.* 1983). Much attention has been paid to this Tet-inducible system because of its non-plant background, which makes it possible to control target genes with a signal transduction system that is not present in plants (Moore *et al.* 2006).

The Tet-inducible system of *Escherichia coli* was first modified as a Tet-on system to regulate eukaryotic gene expression in tobacco by Gatz and Quail (1988). In this system, the sequence flanking the TATA box of *Cauliflower mosaic virus* (CaMV) 35S promoter is substituted by several TetO sequences, and TetR is expressed constitutively to

Received 11 July, 2016 Accepted 28 October, 2016
Correspondence ZHANG Xing-guo, Tel: +86-23-68250974, Fax: +86-23-68251274, E-mail: zhangxg63@163.com

doi: 10.1016/S2095-3119(16)61514-9

suppress this promoter. Thus the expression of the reporter gene is repressed or induced when Tet is absent or present. But this system has been shown to have some defects such as serious leaky expression, weak expression intensity, and toxic effects of TetR when expressed highly in *Arabidopsis*, tomato, and other plants (Corlett *et al.* 1996; Padidam 2003). Thereafter, a Tet-off gene expression system was developed first for mammalian cells (Gossen and Bujard 1992) and then for plants (Weinmann *et al.* 1994). In this system, a Tet-controllable transcriptional activator TetR-VP16 that was created by fusing TetR with the C-terminal activating domain of virion protein 16 (VP16) of the herpes simplex virus, is controlled by a constitutive promoter, and the expressed TetR-VP16 binds to a TetO containing promoter, Top10, to activate the transcription of a target gene. Repression of TetR-VP16-induced target gene expression is achieved by applying low concentrations of Tet (Weinmann *et al.* 1994). The Tet-off expression system based on the TetR-VP16/Top10 construct allows active and stringent control of transgene expression (Böhner *et al.* 1999; Love *et al.* 2000), but unlike its derivative constructs in animals (Lai *et al.* 2004; Karasaki *et al.* 2009; Jin *et al.* 2014), it has not been widely exploited in plants other than in tobacco (Borghi 2010; Bortesi *et al.* 2012).

The use of the Tet-off system in plants has some potential advantages, owing to Tet being absent in natural plants, being able to work at low concentrations and having only indiscernible secondary effects on plants (Weinmann *et al.* 1994). What has obstructed its application is mainly the toxicity of the over-expressed TetR-related transactivator to certain plants and the tedious process of repeatedly replenishing Tet to turn off the constitutively active system as Tet has a short half-life in plants (Corlett *et al.* 1996; Wang *et al.* 2003). To circumvent such problems, a novel Tet-off system was herein constructed, in which a heat shock (HS)promoter was employed to control a chimeric transactivator gene *TetR-HSF*. This system has been characterized for the effectiveness of heat induction and Tet repression by detecting the expression levels of the *TetR-HSF* and *β-glucuronidase* (*GUS*) reporter genes. The new Tet-off and HS-on (Tet-off/HS-on) gene expression system avoids the high-level expression of the TetR-HSF transactivator and the usage of Tet in non-inducing conditions; therefore, it could provide an economical and practical tool in transgenic plant studies to improve agronomic traits or to produce biopharmaceutical proteins. The transgene can be kept nearly silent under non-inducing temperatures, and HS induction of the transgene can be prevented with application of Tet in summer when transgene expression is unnecessary or harmful during the early growth period of plants, tissues, or cells. In the absence of Tet, the transgene can be highly induced under HS conditions in the laboratory or in the field.

2. Materials and methods

2.1. Gene cloning and expression constructs

A TetO containing promoter Om35S was synthesized by GeneRay Biotechnology Co. (Shanghai, China) (Fig. 1-A and B). This Tet-responsive promoter contains four TetO sequences upstream of the −46 bp minimal core region of the CaMV 35S promoter. The four TetOs form an array that mimicks the structure and distribution of the HS elements (HSE) in the HS promoter HSP70m (Zheng *et al.* 2016). In HSP70m, a HSE sequence “GAATCTTCCAGAACTTTC” replaces the TetO1 and TetO2 tandem sequence that is shown in Fig. 1-A and B. A vector pGH-Om35S-Pro carrying the Om35S promoter (provided by GeneRay Biotechnology Co.) was digested with *Hin*dIII and *Xba*I to obtain a 219 bp fragment that was then used to replace the HSP70m promoter in pHSP70m-GUS (Zheng *et al.* 2016) to construct the binary vector pOm35S-GUS, which carries an *Om35S::GUS::Tnos* (*Om35S::GUS*) expression cassette within the T-DNA region (Fig. 1-C).

To construct the chimeric transactivator gene *TetR-HSF*, a 635 bp fragment of *TetR* (GenBank J01830) lacking the translation stop codon was amplified from the genomic DNA of *E. coli* strain XL1-Blue using the primer pairs TetR-F (5′-CCTAGGAATTAATGATGTCTAGATTAG-3′) and TetR-R (5′-GGATCCACTTTCACATTTAAGTTG-3′). Meanwhile, a 1 028 bp fragment of the 3′-terminal transactivation domain of the HS transcription factor (HSF) gene *AtHsfA1a* (GenBank At4g17750; Nover *et al.* 2001) was also amplified from the genomic DNA of *Arabidopsis thaliana* (Columbia ecotype) using the PCR primer pair HSF-F (5′-AGATCTCAGCAATTATCTCAGGGTCAAGG-3′) and HSF-R (5′-GAGCTCTAGTGTTCTGTTTCTGATGTGAG-3′). The above two fragments were cloned separately into a pMD18-T vector (TaKaRa, Dalian, China) through a T/A-end cloning method to obtain plasmids pMD-ΔTetR and pMD-ΔHSF. The plasmid pMD-ΔHSF was digested with enzymes *Bgl*II and *Sac*I, and the resulting 1 018 bp ΔHSF containing fragment was then inserted into the *Bam*HI and *Sac*I sites of pMD-ΔTetR in order to yield pMD-TetR-HSF, which contains the *TetR-HSF* gene fusion. The pMD-TetR-HSF plasmid was subjected to restriction digestion by both *Avr*II and *Sac*I to obtain a 1 647 bp *TetR-HSF* containing fragment, which was then inserted into the *Xba*I and *Sac*I sites to replace the *GUS* gene in pHSP70m-GUS. The resulting plasmid is pHSP70m-TetR-HSF, which carries the chimeric gene *HSP70m::TetR-HSF::Tnos* (*HSP70m::TetR-HSF*). Lastly, the plasmid pHSP70m-TetR-HSF was digested with *Pst*I to obtain a 9 324 bp fragment and recombined with the 2 879 bp Om35S-GUS containing *Pst*I-digested fragment of pOm35S-GUS to gen-